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(12) UK Patent Application (19) GB (11) 2 211 204 (13) A

(43) Date of A publication 28.06.1989

(21) Application No 8823920.7

(22) Date of filing 12.10.1988

(30) Priority data
(31) 62262692 (32) 20.10.1987 (33) JP

(71) Applicant
Oji Paper Co Ltd

(Incorporated in Japan)

7-5 Ginza 4-chome, Chuo-ku, Tokyo, Japan

(72) Inventors
Kazuya Ito
Naomi Tachi
Masaru Shibata

(74) Agent and/or Address for Service
Withers & Rogers
4 Dyer's Buildings, Holborn, London, EC1N 2JT, United Kingdom

(51) INT CL'
C12N 15/00 5/00

(52) UK CL (Edition J)
C6F FHC2 F106
C3H HB7T
C6Y Y1055 Y420 Y501 Y503

(56) Documents cited
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(58) Field of search
UK CL (Edition J) C3H HB7T, C6F FHC2
INT CL' C12N 5/00 15/00
On-Line 'Dialog' (Biotech, Derwent WPI)

(54) **Process for production of plant transformant**

(57) A process for the production of a plant transformant comprising the steps of (1) preparing a mass of shoot primordium from a plant to be transformed, (2) dividing the mass of shoot primordium into small sections, (3) coculturing the small sections of the mass of shoot primordium with cells of *Agrobacterium tumefaciens* in a liquid medium to transform the shoot primordia with a Ti plasmid contained in the cell of *Agrobacterium tumefaciens* , and (4) regenerating a new plant from the transformed mass of shoot primordium; and a process for the production of a plant transformant comprising the steps of (1) preparing a mass of shoot primordium from a plant to be transformed, (2) dividing the mass of shoot primordium into small sections, (3) culturing the small sections of the mass of shoot primordium in a liquid medium, while shaking, to prepare mass of shoot primordium, (4) dividing the mass of shoot primordium obtained in the step (3) into small sections; (5) coculturing the small sections of the mass of shoot primordium obtained in step (4) with cells of *Agrobacterium tumefaciens* in a liquid medium to transform the shoot primordia with Ti plasmid contained in the cells of *Agrobacterium tumefaciens*, and (6) regenerating a plant from the transformed mass of shoot primordium.

claim 1 & 2

GB 2 211 204 A

Fig. 1

Fig. 2

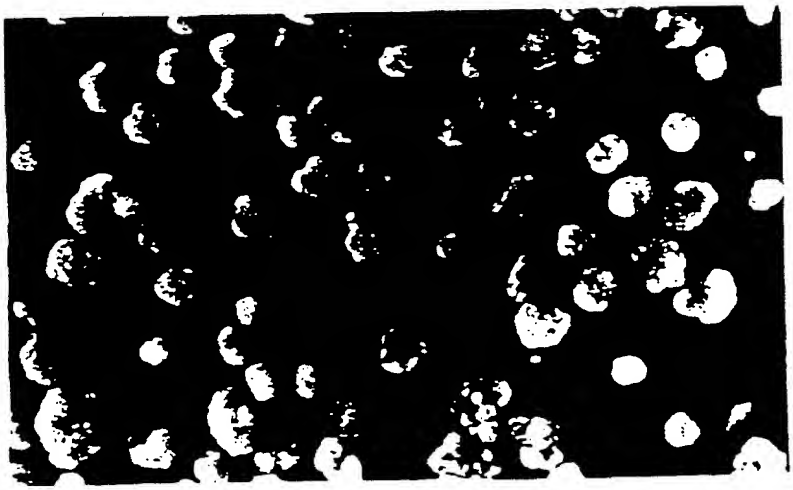

Fig. 3

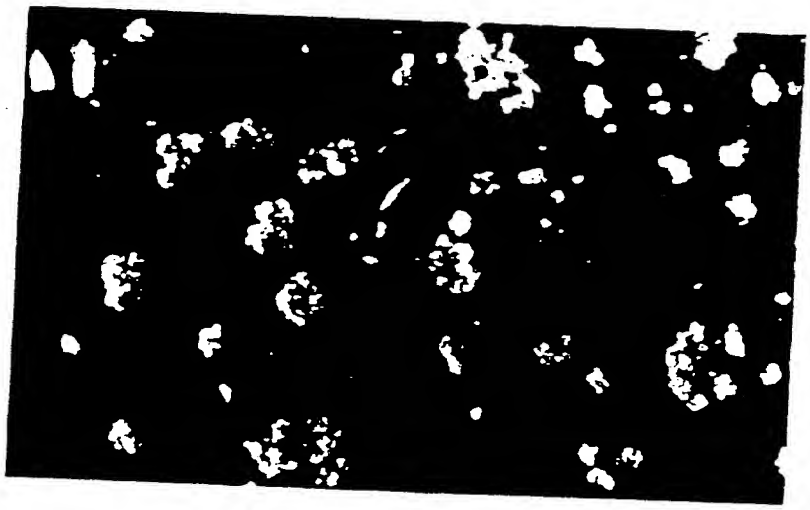

Fig. 4

Fig. 5

PROCESS FOR PRODUCTION OF PLANT TRANSFORMANT

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention generally relates to a process for production of a plant transformant. More specifically, it relates to a process for generating a transformation of plants through shoot primordia.

2. Description of the Related Art

Known techniques for producing a plant transformant, using the infectiousness of Agrobacterium tumefaciens in which the Ti plasmid was contained (tumor-inducing factor) include a derect inoculation method, Matsumoto T. and Machida I., Plant Mol. Biol. Rep. (1986) 4:42 - 47; a leaf disk method, Horch, R.B. et al., Science (1985) 227:1229 - 1231; and McCormick, S. et al., Plant Cell Reports (1986) 5:81 - 84, a co-culturing method with protoplasts, Marton,L. et al., Nature (1979) 277:129 - 131; Wullems, G.T. et al., Proc. Natl. Acad. Sci. USA (1981) 78:4344 - 4348; and Horsch, R.B. et al., Science (1984) 223:496 - 498.

In the direct inoculation method, young seedlings, more suitably, which are cultured aseptically, are pierced with needles on which freshly cultured Abrobacterium tumefaciens adheres, to introduce the Ti plasmid into the plant cells, which are then regenerated to new plants.

In the leaf disk method, a leaf disk is prepared from a leaf, using a paper punch, the disk is soaked in a culture broth of Agrobacterium tumefaciens to introduce an exogenous gene in the Ti plasmid into plant cells, and the transformed plant cells are then regenerated as a new plant.

Although the procedures of the above mentioned two methods are simple, the methods are disadvantageous in that the formation of callus and the regeneration of a plant from the callus take a long time.

In contrast to the above-mentioned methods wherein a plant tissue is infected with the Agrobacterium tumefaciens, in the co-culturing method with protoplasts, protoplasts are infected with the Agrobacterium tumefaciens. Namely, protoplasts are prepared from leaf cells or cultured plant cells, the protoplasts are incubated until the onset of cell division, and at that point, Agrobacterium tumefaciens cells are added to the protoplast culture, and the microbial cells and the plant protoplasts are cocultured to introduce the Ti plasmid in the microbial cells into the plant protoplast. Although this method provides a large number of transformants, the conditions for the protoplast culture and the plant regeneration differ greatly according to the plant species or varieties to which this method is applied, and thus this method can be used only for limited kinds of plants. For example, when this method is to be applied to woody plants, it can be applied to only a very few plants, including, for example, poplar and Citrus.

Recently, various processes for the propagation of plants have been developed. For example, for the propagation of annual plants other than woody plants, a "shoot primordium" method was proposed (Tanaka R. and Ikeda H., Jpn. J. Genet. Vol. 58, 65-70, 1983; Japanese Unexamined Patent Publication No. 59-132822; and Japanese Unexamined Patent Publication No. 59-132823). The "shoot primordium" method, is applied to annual plants such as water-melon, maize, rice, morning glory, Swertia, Papaver and the like, and is also applicable to perennial woody plants such as poplar and eucalypt (Japanese Unexamined Patent Publications No. 61-64800 and No. 62-55020).

Nevertheless, th application of the shoot primordium method to the Ti plasmid-mediated-plant transformation, i.e., a process for plant transformation comprising a combination of the shoot primordium method

and the Ti plasmid method is not known.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides a process for production of a plant transformant using a combination of the shoot primordium method and the Ti plasmid-mediated transformation method, which process can be easily carried out, can be applied to various kinds of plants, and can provide a large number of transformed plants in a short time.

More specifically, the present invention provides a process for the production of a plant transformant comprising the steps of:

(1) preparing a mass of shoot primordium from a plant to be transformed;

(2) dividing the mass of primordium into small sections;

(3) coculturing the small sections of the mass of shoot primordium with cells of Agrobacterium tumefaciens, in a liquid medium, to transform the shoot primordia with Ti plasmid contained in the cells of Agrobacterium tumefaciens; and

(4) regenerating a new plant from the transformed mass of shoot primordium.

The present invention also provides a process for the production of a plant transformant comprising the steps of:

(1) preparing a mass of shoot primordium from a plant to be transformed;

(2) dividing the mass of shoot primordium into small sections;

(3) culturing the small sections of the mass of shoot primordium in a liquid medium, while shaking, to prepare mass of shoot primordium;

(4) dividing the mass of shoot primordium obtained in step (3) into small sections;

(5) coculturing the small sections of the mass of shoot primordium obtained in step (4) with cells

of Agrobacterium tumefaciens, in a liquid medium, to transform the shoot primordia with Ti plasmid contained in the cells of Agrobacterium tumefaciens; and

(6) regenerating a new plant from the transformed mass of shoot primordium.

The novel process of the present invention has the following advantages over the conventional processes:

(1) The conventional techniques wherein parts of a plant are infected with Agrobacterium tumefaciens, i.e., the direct inoculation method and leaf disc method, cannot be applied to plants having a low regeneration ability, because these methods involve callus formation and regenration of a plant from it. However the present invention can be applied to a very wide spectrum of plants and completely isolate and fix transformant, because the regeneration capacity from a mass of shoot primordium is higher enough to product transformants than conventional techniques.

(2) Since the conventional process wherein single cells or protoplasts are infected with Agrobacterium tumefaciens, as in the co-culturing method with protoplasts, must use the essential steps of colony formation, callus formation, and shooting from the callus, the entire process takes a long time. In contrast, since the present process wherein shoot primordia are infected with Agrobacterium tumefaciens does not need the above-mentioned steps, transformed plants can be efficiently isolated and fixed in a short time.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows small sections prepared by finely dividing a mass of shoot primordium of Populus charkowiensis X P. caudia, OP-20;

Fig. 2 shows a mass of shoot primordium of Populus charkowiensis X P. caudia, OP-20 transformed with Agrobacterium tumefaciens and cultured in a hormone-free medium;

Fig. 3 shows untransformed mass of shoot primordium Populus charkowiensis X P. caudia, OP-20 cultured in a hormone-free medium.

Fig. 4 shows transformed shoots of Populus charkowiensis x P. caudina, OP-20 regenrated from the transformed mass of shoot primordium which was selected in a kanamycin-added medium; and

Fig. 5 shows transformed shoots of Crepis capillaris regenrated from the transformed mass of shoot primordium which was selected in a kanamycia-added medium.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

According to the present invention, an herbaceous plant or in woody plant is used to prepare a mass of shoot primordium which is then finely divided into small sections, or optionally, the resulting small sections are additionally cultured in a liquid medium to form a mass of shoot primordium which is again finely divided into small sections. The small sections of the mass of shoot primordium thus prepared are then cocultured with cells of Agrobacterium tumefaciens to transform cells of the shoot primordium sections with a Ti plasmid comprising T-region in which an exogenous gene has been inserted. The transformed mass of primordium is then regenerated into a transformed plant.

Plants to which the present method can be applied include, but are not limited to, evergreen broad-leaved trees such as eucalypt, acacia, caoutchouc, and coffee; deciduous broad-leaved trees such as poplar, paulownia, Quercus, oak, Japanese lacquer; conifers such as pine, Japanese cedar, Japanese cypress, fir, spruce, Japanese larch; fruit trees such as orange, lemon, apple, peach, avocado, kiwi-fruit, persimmon, walnut, grape, fig, almond, and mango; and flowering shrubs or trees such as rose, camellia, ume (Japanese apricot), and cherry. Moreover, in addition to the above-mentioned woody trees, the present method can be applied to herbaceous

plants, for example, flowers, such as petunia and cosmos; and crops such as tobacoo, flax, rice, wheat, tomato, spinach, and soybean.

Preparation of a mass of shoot primordium

Shoot pieces are removed from a plant to be transformed, sterilized in a sterilizing solution, and washed thoroughly with sterilizing water. Under a stereo-microscope, shoot tips containing a pair of leaf primordium and having a length of about 0.5 mm are aseptically removed from the sterilized shoot pieces, and the shoot tips are transplanted to an artificial liquid medium containing inorganic salts, organic salts, and plant growth hormones.

The composition of inorganic and organic salts contained in the artificial liquid medium varies depending on the kind of plant to be treated, but fundamentally is a Gamborg B5 medium (abbreviated as B5 medium hereinafter), or a Murashige-Skoog medium (abbreviated as MS medium hereinafter). The plant growth hormones include auxins such as naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-butylic acid (IBA), phenylacetic acid (PAA), benzofuran-3-acetic acid (BFA), phenyl-butylic acid (PBA) and the like; and cytokinins such as 6-benzylaminopurine (also called benzyladenine) (BA), kinetin, KT-30 (Kyowa Hakko, Japan), zeatin and the like.

A culture is carried out on a gyrated drum placed obliquely at a constant temperature of between 15°C and 30°C, preferably 25°C and 30°C. At a lower temperature, the propagation is delayed; and at too high a temperature, the growth is poor and unstable. The illumination is carried out, preferably continuously, at a str ngth of 2,000 to 20,000 lux using a fluorescent lamp. A higher or lower strength of illumination has an adverse affect on the growth of

a mass of shoot primordium. The culture method on a gyrated drum is essential to ensure a sufficient growth of a mass of primordium; i.e., a culture on agar plate will provide only a poor growth of a mass of shoot primordium.

For the culture, for example, a culturing apparatus (Nippon Ika Kikai Seisakusho, Japan) having a gyrated drum of a diameter of about 100 cm is used. Test tubes containing a medium into which a shoot tip is transplanted are attached to the gyrated drum in such a manner that the tubes are parallel with a rotation axis of the drum, and constantly face in the same direction during the rotation of the gyrated drum. The tubes are illuminated from above. The rotation rate of the gyrated drum is preferably as low as 1 to 10 rpm, since too high a rotation rate provides a large amount of callus, and too low a rotation rate provides a large amount of precocious branching, both resulting in a poor mass of shoot primordium.

The mass of shoot primordium is obtained as described above, and optionally, the thus-prepared mass of shoot primordium is proliferated, as follows: The mass of shoot primordium is homogenized in a homogenizer at 10,000 to 30,000 rpm for 5 to 20 seconds in a washing solution, to prepare small sections of the mass of shoot primordium, and the small sections are washed one to five times in the washing solution. The washing solution is a Gamborg B5 medium or a Murashige-Skoog medium, supplemented with an auxin such as 2,4-dichlorophenoxy-acetic acid (2,4-D), naphthalene acetic acid (NAA), indole-3-acetic acid (IAA) or the like, and a cytokinin such as benzyladenine (BA), kinetin, KT-30, zeatin or the like, and further supplemented with sucrose.

Th small sections of the mass of shoot primordium prepared as described above is optionally transplanted to a medium as described above, and are cultured on a rotary shaker for 12 to 16 hours at a temperature of

20°C to 30°C, under an illumination having a strength of 5,000 to 20,000 lux, using a fluorescent lamp, and while shaking at 80 to 100 rpm, to obtain a large amount of a mass of shoot primordium. The process of proliferation of the mass of shoot primordium is described in more detail in Japanese Patent Application No. 62-204269.

Coculturing of a mass shoot primordium with Agrobacterium tumefaciens

Strains of Agrobacterium tumefaciens used for the present invention are, for example, C58 which is a wild type strain, causes a crown gall formation and makes a plant cell grow on a hormone-free medium and, LBA4404 which is a part of Gus gene fusion system commerciallized from Clontech Laboratories, Inc. USA. In the use of Gus gene fusion system, transformed cells are capable of growing on a medium containing kanamycin. The strain C58 is disclosed by Depicker et al., Molec, Gen. Genel. 188, 425(1982), and deposited with ATCC as No. 3397D.

The Agrobacterium tumefaciens is stored and cultured by a conventional procedure. For example, the A. tumefaciens is stored at -50°C to -80°C, and when to be used, the stored cells are inoculated on a agar plate medium having a conventional composition for bacterial culture, to form single colonies. Cells from the single colony are then inoculated to a liquid medium, and cultured for about one or two days, while shaking, at a temperature of 25°C to 30°C. The cultured broth is the centrifuged to separate the cultured cells of A. tumefaciens, which are then optionally washed.

The bacterial cells are then added, for example, at a concentration of 10^6 - 10^9/ml, to a medium containing the small sections of the mass of shoot primordium prepared as described above, and the coculturing of the shoot primordium sections with the A. tumefaciens cells is carried out at 20°C to 30°C for about 48 hours, while shaking at 50 to 100 rpm, to transform the shoot pri-

mordia with the Ti plasmid contained in the bacterial cells. The medium used for the coculturing is usually a Gamborg B5 medium or Murashige-Skoog medium supplemented with an auxin such as naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA) or the like, and a cytokinin such as benzyladenine (BA), KT-30, kinetin, zeatin or the like.

Selection of a transformed mass of shoot primordium

After the coculturing, antibiotics such as vancomycin, carbenicillin, tetracycline, and/or ampicillin are added to the culture, and the whole is incubated at a temperature of 20°C to 30°C for 2 to 14 days.

After the incubation, a mass of shoot primordium is transferred to a hormone-free B5 or MS medium in the case of coculturing with C58 strain, and to a kanamycin added B5 or MS medium in the case of coculturing with LBA4404 strain. Both are cultured at a temperature of 20°C to 30°C for 7 to 14 days, under an illumination having a strength of 3,000 to 5,000 lux, using a fluorescent lamp, while shaking at 50 to 100 rpm. During this culturing only the transformed mass of shoot primordium will grow; and untransformed mass of shoot primordium is reduced in size and suffer a change in color of from green to brown. Accordingly, the transformed mass of shoot primordium is easily selected, due to their different size and color.

Regeneration of a plant from a transformed mass of shoot primordium divided from coculturing with LBA4404

The mass of shoot primordium grown and selected as described above are transferred to a liquid shooting medium having a composition similar to that used for the formation of mass of shoot primordium exact for addition of Kanamycin, and cultured on an agar plate at 15°C to 30°C, under an illumination having a strength of 1,000 to 4,000 lux, for 20 to 30 days, to form a large number of small shoots. Next, the shoots are separated and

transferred to a rooting medium, in which the shoots are rooted, and thus entire small plants are obtained. Note, a period of about three months must elapse from the start of the culture on an agar plate before an entire plant is formed.

Note, in some cases, the transformed mass of shoot primordium contains, in addition to the transformant, untransformed shoot primordia. In this case, a conventional individual selection process must be carried out to eliminate the untransformed plants.

The present invention will now be further illustrated by, but is by no means limited to, the following example.

Example 1

Plant used for experiment

Populus charkowiensis X P. caudia, OP-20

Agrobacterium used for experiment

C58 strain

Preparation of shoot primordia

Top pieces having a length of about 20 mm were cut from a green branch of an actively growing poplar and sterilized with 70% ethanol for 5 minutes, with 7-fold diluted sodium hypochlorite solution for 15 minutes, and then washed with sterilized water. Next, in an eseptic condition, shoot tips including a growing point and having a length of about 0.5 mm were removed from the sterilized pieces, using tweezers and a surgical knife under a stereomicroscope. The removed shoot tips were then transplanted to the Gamborg medium having the composition set forth in Table 1.

Table 1

Medium for Poplar*

Component	Concentration (mg/l)
$NaH_2PO_4 \cdot H_2O$	150
KNO_3	2,500
$(NH_4)2SO_4$	134
$MgSO_4 \cdot 7H_2O$	250
$CaCl_2 \cdot 2H_2O$	150
Fe-EDTA	40
$MnSO_4 \cdot 4H_2O$	10
H_3BO_3	3
$ZnSO_4 \cdot 7H_2O$	2
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
KI	0.75
nicotinic acid	1
thiamine-HCl	10
pyridoxine-HCl	1
myo-inositol	100
sucrose	30,000
naphthaleneacetic acid	0.05
6-benzylaminopurine	0.4
pH	5.6

* Basal medium is Gamborg B5.

Culture was carried out in 25 ml of the medium of Table 1 in a test tube having a diameter of 30 mm and a length of 200 mm, at a temperature of 28°C, illumination intensity of 2,000 to 20,000 lux, and a rotation rate of 2 rpm.

At the 40th day from the start of culturing, a green mass of shoot primordium having a diameter of about 10 mm was obtained. After three weeks, the growing mass of shoot primordium was then divided into mass of shoot primordium having a diameter of about 5 to 10 mm, and the divided mass of shoot primordium was transplanted to a freshly prepared medium having the same composition as described above and cultured for two weeks. To the proliferating mass of shoot primordium was added, in an amount of 15 ml per 1 g of the mass of shoot primordium, a washing solution which comprised the medium described in Table 1. The whole was then homogenized in a homogenizer at 20,000 rpm for 10 seconds, to prepare small sections having a size of about 1 mm from the mass of shoot primordium. The homogenate was filtrated through a nylon mesh to recover these small sections, which were then washed twice with the washing solution.

To 1 g of the washed small sections of the mass of shoot primordium was added 100 ml of the above mentioned medium, and the mixture was placed in a 500 ml conical flask. Culture was carried out at 27°C, under 12 hours of photoperiod having a strength of 10,000 lux while shaking at 100 rpm. A photograph of the culture is shown in Fig. 1.

Coculture of small sections of the mass of shoot primordium with cells of A. tumefaciens (C58 strain)

Cells of Agrobacterium tumefaciens C58 stored at -80°C were inoculated to a Lennox medium (peptone 10 g/l, yeast extract 5 g/l, NaCl 10 g/l, agar 15 g/l pH 7.2), and cultured at 28°C under an illumination

having a strength of 3,000 lux, to form colonies. A single colony was selected and inoculated to 50 ml of a liquid medium having the same composition as described above, except that agar was not added, in a 500 ml conical flask, and cultured overnight at 28°C. The culture broth was centrifuged at 4,600 rpm for 10 minutes to separate the cultured cells, which were then suspended at a concentration of 2 x 10^8 cell/ml.

Two ml of the bacterial suspension was added to 100 ml of the mass of the culture of shoot primordium as described above, and coculturing was carried out for 48 hours at 28°C while shaking at 100 rpm.

Selection of a transformed mass of shoot primordium

The coculture prepared as described above was filtrated to recover the mass of shoot primordium, which was then washed. The mass of shoot primordium was then cultured in a medium, which was the same as the above-mentioned medium (Table 1) except that 250 mg/l of vancomycin and 200 mg/l of carbenicillin were added, at 25°C for 10 days while shaking at 100 rpm.

Next, the mass of shoot primordium thus obtained was transferred to a medium, which was the same as the above-mentioned medium (Table 1) except that the hormones were omitted (hormone-free medium), and cultured for 20 days 28°C under an illumination having a strength of 5,000 lux while shaking at 100 rpm.

As a result, a remarkable growth of green transformed mass of shoot primordium occurred. A photograph of the transformed mass of shoot primordium is shown in Fig. 2. Conversely, the untransformed mass of shoot primordium did not grow in the above-mentioned hormone-free medium, and suffered a change in color of from green to brown. Accordingly, the transformed mass of shoot primordium was easily selected due to the difference in color and size thereof. Figure 3 is a photograph showing the untransformed mass of shoot primordium cultured in the hormone-free medium.

Example 2

Plant used for experiment

Populus charkowiensis x P. caudina, OP-20

Agrobacterium used for experiment

LBA4404 strain (containing pB1121 plasmid on which kanamycin resistant gene are located)

Preparation of shoot primordia

For preparation of a mass of shoot primordium, the same procedure as describe in Example 1 was repreated.

Coculture of small sections of a mass of shoot primordium with cells of A. tumefaciens (LBA4404 strain)

Culture of the small section was carried out according to the same procedure as described in Example 1.

Selection of a transformed mass of shoot primordium

The coculture prepared as described above was filtrated to recover the mass of shoot primoridum, which were then washed. The mass of shoot primordium were then cultured in a medium, which was the same as the medium (Table 1) except for addition of 500mg/l of carbenicillin and 50mg/l of kanamycin, at 25° for 14 days, while shaking at 100 rpm.

Regeneration from a selected mass of shoot primordium

The transformed mass of shoot primordium was able to grow on a kanamycin added medium but the untransformed mass of shoot primoridium was not able to grow on the same medium and its color changed brown in this medium.

The transformed mass of shoot primordium was trnasferred to the regenration medium which was the same as the selection medium except for hormonal concentration and addition of agar (0.6%). Hormonal concentration of the reg neration medium was 0.02 mg/l of NAA and 0.02 mg/l of BA. Transformed shoots were regenerated from the transformed mass of shoot primordium after 20

days of culture. Fig. 4 is a photograph showing the renegeration transformed shoots.

Example 3

Plant used for experiment

Crepis capillaris

Agrobacterium used for experiment

LBA4404 strain (containing pB1121 plasmid on which kanamycin resistant gene are located)

Preparation of shoot primordia

A mass of shoot primordium of Crepis were prepared according to the literature (Tanaka, R. & H.Ikeda, Jpn. J. Genet., (1983) 58:65 - 70; Japanese Unexamined Patent Publication, 59-132822).

Co-culture of small sections of a mass of shoot primordium with cells of A. tumefaciens (LBA4404 strain)

Culture was carried out according to the same procedure as described in Example 1 except for composition of the medium.

Selection of a transformed mass of shoot primordium

The coculture prepared as described above was filtrated to recover the mass of shoot primordium, which were then washed. The mass of shoot primordium was then culture in a medium, which was the same as the medium (Table 2) except for addition of 500 mg/l of carbenicilline and 50 mg/l of kanamycin, at 25°C for 4 days, while shaking at 100 rpm.

Table 2
Medium for Crepis*

Component	Concentration (mg/l)
KH_2PO_4	170
KNO_3	1,900
NH_4NO_3	1,650
$MgSO_4 \cdot 7H_2O$	370
$CaCl_2 \cdot 2H_2O$	440
$MnSO_4 \cdot 4H_2O$	22.3
H_3BO_3	6.2
$ZnSO_4 \cdot 7H_2O$	8.6
$Na_2MoO_4 \cdot 7H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
KI	0.83
$FeSO_4 \cdot 7H_2O$	27.8
Na_2EDTA	37.8
nicotinic acid	0.5
thiamine-HCl	1
pyridoxine-HCl	0.5
glycine	2
myo-inositol	100
sucrose	30,000
naphtalene acetic acid	0.5
6-benzylaminopurine	0.5
pH	5.6

* Basal medium is Murashige and Skoog

Regeneraton from a selected mass of shoot primordium

The transformed mass of shoot primordium was able to grow on a kanamycin added medium, but the untransformed mass of shoot primordium was not able to grow on the same medium and its color changed brown in this medium.

The transformed mass of shoot primordium was transferred to the regeneration medium which was the same as the selection medium except for hormonal concentration and addition of agar (0.6%). Hormonal concentration of the regeneration medium was 0.02 mg/l of BA. Transformed shoots were regenerated from the transformed mass of shoot primordium after 14 days of culture. Fig. 5 is a photograph showing the regenerating transformed shoots.

As described above, the process for the regeneration of a plant transformant according to the present invention can be applied to various kinds of plants, simply and with a higher transformation ratio, in comparison to conventional transformation methods, and is original and very useful.

CLAIMS

1. A process for production of a plant transformant comprising the steps of:

(1) preparing a mass of shoot primordium from a plant to be transformed;

(2) dividing the mass of shoot primordium into small sections;

(3) coculturing the small sections of the mass of shoot primordium with cells of Agrobacterium tumefaciens in a liquid medium to transform the shoot primordia with a Ti plasmid contained in the cells of Agrobacterium tumefaciens; and

(4) regenerating a new plant from the transformed mass of shoot primordium.

2. A process for production of a plant transformant comprising the step of:

(1) preparing a mass of shoot primordium from a plant to be transformed;

(2) dividing the mass of shoot primordium into small sections;

(3) culturing the small sections of the mass of shoot primordium in a liquid medium, while shaking, to prepare mass of shoot primordium,

(4) dividing the mass of shoot primordium obtained in step (3) into small sections;

(5) coculturing the small sections of the mass of shoot primordium obtained in step (4) with cells of Agrobacterium tumefaciens in a liquid medium to transform the shoot primordia with Ti plasmid contained in the cells of Agrobacterium tumefaciens; and

(6) regenerating a new plant from the mass of transformed shoot primordium.

3. A process for production of a plant transformant according to claim 1 or 2, wherein the coculturing is carried out for 24 to 48 hours, at a temperature of 20°C to 30°C while shaking at 50 to 100 rpm.

4. A process for production of a plant transformant according to claim 1 or 2, wherein the liquid medium for the coculturing is a Gamborg B5 medium or Murashige-Skoog medium supplemented with plant hormones.

5. A process for production of a plant transformant according to claim 1 or 2, wherein the liquid medium of the coculturing is a Gamborg B5 medium or Murashige-Skoog medium supplemented with an auxin and cytokinin.

6. A process for production of a plant transformant according to claim 1 or 2, which process further comprises, between the steps of coculturing and the regeneration of a plant, the step of eliminating bacterial cells by incubating the transformed mass of shoot primordium in a medium containing antibiotics.

7. A process for production of a plant transformant according to claim 1 or 2, which process further comprises, between the step of the coculturing and the regeneration of a plant, the step for eliminating untransformed mass of shoot primordium by incubating the mass of shoot primordium from the coculturing step in a hormone-free medium or a kanamycin-added medium.